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MINIREVIEW

Ambler Class A Extended-Spectrum β-Lactamases in *Pseudomonas aeruginosa*: Novel Developments and Clinical Impact

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The so-called clavulanic acid-inhibitory extended-spectrum β-lactamases (ESBLs) belong mostly to class A of the Ambler classification scheme (1) and confer resistance to at least several expanded-spectrum cephalosporins (19, 28). They have been extensively reported in members of the family *Enterobacteriaceae* since the early 1980s, whereas they have been described in *Pseudomonas aeruginosa* only more recently.

These enzymes are either of the TEM and SHV types, which are also well known in the *Enterobacteriaceae*; of the PER type, mostly originating from Turkish isolates; of the VEB type from Southeast Asia; or, more recently, of the GES and IBC types, which have been reported from France, Greece, and South Africa (14, 25, 27, 30–32, 34, 35, 42, 43, 45, 51). These five types of enzymes are remotely related from a genetic point of view, although they share similar hydrolytic profiles. Recent studies indicate that dissemination of the genes for these β-lactamases may play an important role in the spread of antibiotic resistance and may limit future choices of antibiotic regimens for the treatment of life-threatening infections due to ESBL-producing *P. aeruginosa* (13, 17, 45). This minireview focuses on the epidemiology, substrate profile, genetic background, detection, and clinical consequences of class A ESBLs in *P. aeruginosa*.

EPIDEMIOLOGY

As summarized in Table 1, these enzymes have so far been found in a limited number of geographic areas, suggesting that, at least in several cases, some of these β-lactamase genes may possess a specific ecological niche. The SHV-type ESBLs have been identified in very rare isolates of *P. aeruginosa*; SHV-2a has been identified in France, whereas SHV-5 and SHV-12 were detected in Thailand (8, 31). Except for the SHV-12 producer, these isolates were nosocomial strains; the SHV-12 producer was isolated from a clinical sample from an outpatient of a Thai hospital (8).

The TEM-type enzymes described in *P. aeruginosa*, namely, TEM-4, TEM-21, TEM-24, and TEM-42, have been reported in rare isolates from France (3, 13, 25, 30, 42). A French survey indicated that only 10% of ticarcillin-resistant *P. aeruginosa* isolates (i.e., 1.9% of *P. aeruginosa* isolates) produce a TEM-type β-lactamase, whereas other narrow-spectrum β-lactamases (OXA and CARB) are more frequently encountered in that species (3). Conversely, the TEM-type enzymes are widely distributed among the *Enterobacteriaceae*, whereas OXA-type and CARB-type β-lactamases are rare (23). The rarity of reports of *P. aeruginosa* strains harboring genes for TEM- and SHV-type enzymes may have several explanations. First, the rarity of narrow-spectrum TEM-type enzymes may limit antibiotic selection of TEM- and SHV-type enzymes with an expanded spectrum of hydrolysis. Second, a high prevalence of chromosome-encoded oxacillinase and carbenicillinase genes may explain why narrow-spectrum enzymes of the TEM type are rare in *P. aeruginosa*. Indeed, several oxacillinases (OXA-2 and OXA-10 derivatives and OXA-18) that have extended substrate profiles, including extended-spectrum cephalosporins, have been reported in *P. aeruginosa* (9, 38). Third, expression of the chromosome-encoded cephalosporinase of *P. aeruginosa* may be up-regulated (derepressed) and may thereby be a more convenient way for acquisition of resistance to expanded-spectrum cephalosporins (20), without the need for expansion of its genetic repertoire. It is likely that the genes for the TEM- and SHV-type ESBLs in *P. aeruginosa* originated in *Enterobacteriaceae*, from which the genes were passed by gene transfer. This has been shown for the sequence of TEM-24 (25) and the downstream-located DNA sequences of the chromosome of *P. aeruginosa* RP-1, which produces SHV-2a, which were found to be identical to those reported to be plasmid encoded in a *Klebsiella pneumoniae* isolate (31, 39). Differences in the replication origins of plasmids from *Enterobacteriaceae* and *P. aeruginosa* may, however, limit such intergeneric transfers. Additionally, the difficulty of detection of TEM- and SHV-type ESBLs in the clinical laboratory may underestimate their true prevalence in *P. aeruginosa*.

The β-lactamase PER-1 was the first ESBL identified and fully characterized in *P. aeruginosa*, which occurred in 1993 (34, 35). It shares only 18 to 20% amino acid identity with the TEM- and SHV-type ESBLs (Table 2; Fig. 1). It was found in a *P. aeruginosa* isolate from a Turkish patient hospitalized in the Paris, France, area in 1991 (34). A subsequent study on the distribution of the bla_{PER-1} gene revealed that it is widespread in Turkey, with PER-1 being identified in up to 46% of *Acinetobacter* strains and 11% of *P. aeruginosa* isolates analyzed in a nation-based survey performed over a 3-month period in

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TABLE 1. Ambler class A extended-spectrum β-lactamases known in *P. aeruginosa*

β-Lactamase	Genetic support[a]	Country of first isolation	Yr of first isolation	Other countries of isolation	Reference(s)
VEB-1	C, P, I	France	1998	Thailand, India,[b] China[b]	8, 17, 32, 51
VEB-1a	C, I	Kuwait	1999		43
VEB-1b	C, I	Kuwait	1999		43
VEB-2	C, I	Thailand	1999		17
PER-1	C	France	1991	Turkey, Italy, Belgium	10, 12, 24, 34, 53, 54
SHV-2a	C, P	France	1995	Thailand, Poland[b]	8, 31
SHV-5	P	Thailand	1994–1996	Greece[b]	8
SHV-12	C	Thailand	1994–1996		8
TEM-4	P, C	France	1996		42
TEM-21	C	France	1997		13
TEM-24	P	France	1998		25
TEM-42	P	France	1992		30
GES-1	C, I	France	1999		14
GES-2	P, I	South Africa	2000		44, 45
IBC-2	C, I	Greece	1998		27

[a] C, chromosomal location; P, plasmid borne; I, integron-borne.
[b] P. Nordmann, personal data.

1999 (54). PER-1 was identified in up to 38% of ceftazidime-resistant *P. aeruginosa* isolates, with ribotyping results indicating the spread of different clones (54). Since screening for the bla_{PER-1} gene has not been performed in *P. aeruginosa* isolates originating from countries neighboring Turkey, such as Syria, Iran, and Iraq, no current data exist on the true prevalence of PER-1 in the Middle East.

It is possible that the spread of PER-1 in Western Europe may be mostly related to the immigration of Turkish nationals. Interestingly, although it has been reported in several enterobacterial species including community-acquired pathogens such as *Salmonella* spp. (53), the PER-1 β-lactamase mostly seems to be expressed by *P. aeruginosa* and *Acinetobacter* sp. isolates in Turkey (52, 54). A large nosocomial outbreak of PER-1-producing *P. aeruginosa* that occurred over a 10-month period in a tertiary-care hospital was documented in Varese, Italy (24). During that outbreak, a total of 108 clinical isolates were recovered from 18 patients, reflecting the propensity of *P. aeruginosa* to widely colonize hospitalized patients. In that case, apart from the β-lactam resistance phenotype conferred by PER-1, epidemic strains were resistant to several disinfectants, including chlorhexidine, iodide povidone, and toluene-*p*-sulfochloramide (24). Control of the outbreak was obtained by implementing strict hygienic measures, carbapenem therapy, and disinfection of decubitus ulcers and surgical wounds with Mercurochrome (merbromin) or silver nitrate solutions (24). As a result of increased rates of carbapenem consumption, selection of several carbapenem-resistant organisms took place in the nosocomial environment, including an OprD-defective *P. aeruginosa* strain, *Stenotrophomonas maltophilia*, and a *Pseudomonas putida* strain producing the class B carbapenemase VIM-1 (24). The same group had reported a *P. aeruginosa* strain that produced the plasmid-mediated β-lactamase VIM-2 together with the PER-1 β-lactamase (12), thus showing that the same *P. aeruginosa* strain may produce two unrelated β-lactamases, both with expanded-spectrum hydrolysis. Recently, another *P. aeruginosa* strain that produced PER-1 has been isolated from a patient hospitalized in Clermont-Ferrand, in the central part of France (11). Indeed, the latter patient had previously been hospitalized in Strasbourg, in the eastern part of France, where the patient might have been in contact with hospitalized Turkish patients (D. Sirot, personal communication). A pseudo-outbreak has been also reported in Belgium (10), revealing the obstacles that face investigators when they are searching for the source of multiresistant *P. aeruginosa* isolates. Although no mention is made about the antibiotic regimen used to treat the infected patients, this pseudo-outbreak was successfully terminated by decontamination of a side-room urine densitometer (10).

TABLE 2. Percent amino acid identity between representatives of each type of Ambler class A ESBL identified in *P. aeruginosa*

ESBL	% Amino acid identity			
	TEM-4	SHV-2a	VEB-1	PER-1
SHV-2a	63			
VEB-1	19	21		
PER-1	18	20	38	
GES-1	31	30	19	23

Another unrelated ESBL from *P. aeruginosa*, i.e., the β-lactamase VEB-1, was originally identified in *Escherichia coli* and *Klebsiella* isolates from a 4-month-old Vietnamese child transferred from Vietnam and hospitalized in France (41). It was distantly related to other class A ESBLs (Table 2; Fig. 1). Subsequent isolation of VEB-1 from *P. aeruginosa* strains from two patients hospitalized in France and transferred from Thailand was documented (32). A study conducted in a university hospital in Thailand (17) revealed that bla_{VEB}-like genes were present in up to 93% of the ceftazidime-resistant isolates, whereas ceftazidime resistance occurred in 24% of *P. aeruginosa* isolates. Similarly, bla_{VEB-1} was widespread in the *Enterobacteriaceae* in the same hospital (18). Another bla_{VEB-1}-like gene, bla_{VEB-2}, was identified during that study, with VEB-2 differing from VEB-1 by only one amino acid change, located outside the active site of the enzyme (17) (Fig. 1). The latest development in the analysis of bla_{VEB-1}-like genes was the isolation of *P. aeruginosa* strains from an intensive care unit of a Kuwaiti hospital harboring bla_{VEB}-like genes, bla_{VEB-1a} and bla_{VEB-1b}, that differed from the bla_{VEB-1} gene by nucleotide substitutions in the DNA sequence encoding the leader pep-

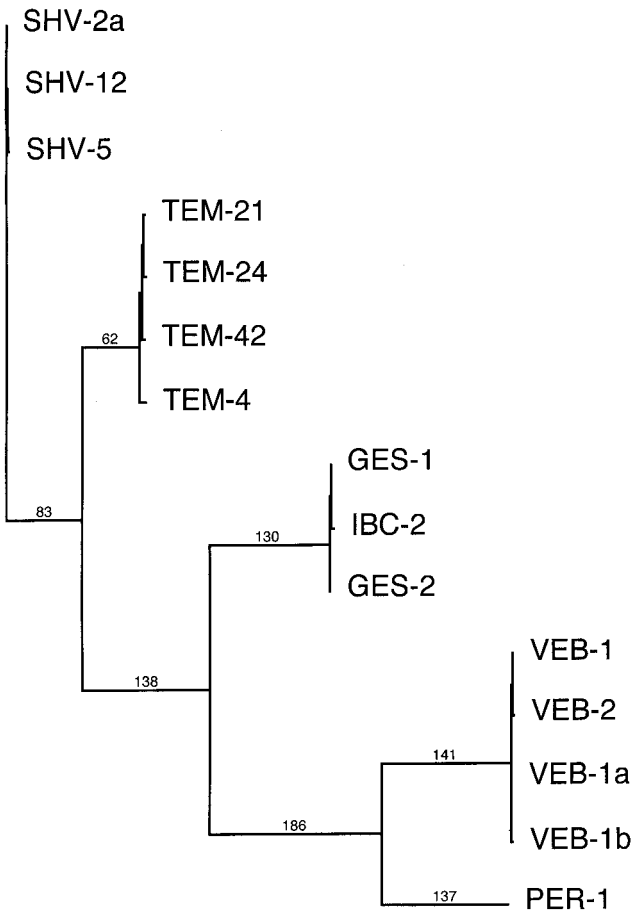

FIG. 1. Dendrogram obtained for class A ESBLs identified in *P. aeruginosa* by parsimony analysis. The alignments used for construction of the tree were carried out with the ClustalW program, followed by minor adjustments to fit the class A β-lactamase scheme (1). Branch lengths are drawn to scale and are proportional to the number of amino acid changes. The number of changes is indicated above each branch. The distance along the vertical axis has no significance.

tide (43) (Table 1). Unpublished data have also identified VEB-1 in *P. aeruginosa* strains in India and China (P. Nordmann, personal data). It is likely that VEB-type enzymes may be isolated mostly from patients coming from or hospitalized in Asia.

Another ESBL, GES-1, was first identified from a French Guiana *K. pneumoniae* strain isolated in Paris (40). Subsequently, bla_{GES-1} was identified from a *P. aeruginosa* isolate in France (14), and the structurally related bla_{IBC-2} gene was isolated from a Greek isolate in Thessaloniki (27). IBC-2 differs by only one amino acid residue (Leu instead of Ala120) from GES-1 and by two residues from IBC-1 (Fig. 1) (16, 27). One of the most interesting developments in research on ESBLs in *P. aeruginosa* is the identification of GES-2, which differs from GES-1 by a single amino acid change, located in the active sites of these enzymes (45) (Fig. 1). GES-2 hydrolyzes not only extended-spectrum cephalosporins but also imipenem, to a minor extent (44). This enzyme was identified in a *P. aeruginosa* strain from a patient hospitalized in the university hospital of Pretoria, South Africa, and was associated with isolates involved in an outbreak that occurred in the same hospital from March to July 2000 (45). Indeed, a single isolate (or clonally related isolates) was identified in eight patients carrying the same plasmid-encoded bla_{GES-2} gene (44). The presence of the $bla_{GES/IBC}$ genes in *P. aeruginosa* and in other gram-negative rods in different countries might indicate the yet undiscovered potential spread of these β-lactamase genes. These results suggest that these ESBL genes might have a wider random distribution than the VEB and PER enzymes.

Another putative but not yet fully characterized ESBL was identified in Tunisia (2, 46), again focusing attention on Mediterranean countries as possible reservoirs of ESBL-producing *P. aeruginosa* isolates. Additionally, other noncharacterized ESBLs have been described from *P. aeruginosa* isolates in Brazil (37) and Poland (55). Although several class A ESBLs are found in *P. aeruginosa* and the *Enterobacteriaceae* (TEM, SHV, VEB, PER, GES/IBC), other ESBLs such as BES-1, TLA-1, and the CTX-M-type enzymes are so far restricted to the *Enterobacteriaceae* (4, 33, 48).

SUBSTRATE PROFILE

The hydrolytic properties of the ESBLs of the TEM type found in *P. aeruginosa* (13, 25, 30, 36, 42) are similar to those of classical TEM-type ESBLs hydrolyzing narrow-spectrum penicillins, extended-spectrum cephalosporins, and the monobactam aztreonam (6, 28). TEM-4 has a substrate profile that mostly includes cefotaxime (Table 3), whereas TEM-42 exhibits a low K_m for ceftazidime (30, 36),

The affinity of the β-lactamase SHV-2a mirrors that of TEM-4 to some extent (Table 3), with a high affinity for the latest cephalosporins developed, such as cefpirome (39). Slight differences in kinetic parameters were found for SHV-5 and SHV-12. The kinetic constants of SHV-5 reveal subtle differences in substrate profiles compared to those of the TEM enzymes, most notably, that of TEM-42.

The non-TEM, non-SHV ESBLs from *P. aeruginosa* also tend to exhibit a fairly broad range of substrate specificities (Table 3). VEB-1 and PER-1 exhibit the substrate profiles typical of classical ESBLs, i.e., high affinities for narrow-spectrum penicillins and narrow- and expanded-spectrum cephalosporins (Table 3). PER-1 in particular exhibits high levels of catalytic activity toward cefotaxime and aztreonam (5), whereas VEB-1 hydrolyzes cefotaxime better than it hydrolyzes ceftazidime (41). These ESBLs have low-level affinities for the carbapenems and are moderately inhibited by clavulanic acid and imipenem. The β-lactamases VEB-1a and VEB-1b have the same substrate specificities as VEB-1, since the distinctive mutations are located in the mature protein sequences outside the putative active site (43). In addition, VEB-1 and PER-1 are well inhibited by cefoxitin, with their K_i values for this β-lactam molecule being 15 and 40 nm, respectively (34, 41).

The β-lactamase GES-1 is peculiar in its low level of catalytic activity, its low affinity for most substrates (40), and its inhibition profile, which includes clavulanic acid and imipenem (Table 3). As opposed to most class A β-lactamases, GES-1 has a high affinity for cefoxitin (Table 3). The latest addition to the GES lineage (GES-2) tends to swing its substrate affinities

TABLE 3. Comparative kinetic parameters for ESBLs found in *P. aeruginosa*[a]

Antibiotic	GES-1			GES-2			PER-1			VEB-1			TEM-4			SHV-2a		
	k_{cat} (s^{-1})	K_m (μM)	k_{cat}/K_m	k_{cat} (s^{-1})	K_m (μM)	k_{cat}/K_m	k_{cat} (s^{-1})	K_m (μM)	k_{cat}/K_m	V_{max}	K_m (μM)	V_{max}/K_m	V_{max}	K_m (μM)	V_{max}/K_m	V_{max}	K_m (μM)	V_{max}/K_m
Benzylpenicillin	2.8	40	70	0.4	4	96	7.2	31.3	230	100	2.8	100	100	NA	NA	33	17	1.9
Amoxicillin	13	200	65	0.7	25.8	26	NA	NA	NA	110	6.0	50	50	NA	NA	NA	NA	NA
Ampicillin	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	47	28	1.7
Ticarcillin	0.3	400	0.7	0.06	13.3	4.5	NA	NA	NA	8	1	22	NA	NA	NA	NA	NA	NA
Piperacillin	8	900	13	0.3	22.8	23	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Cephalothin	179	3,400	52	0.3	3	112	12.4	46	269	700	6.0	325	NA	NA	NA	NA	NA	NA
Cephaloridin	53	2,000	26	0.5	7.7	65	13	237	22	2,300	12	533	232	NA	NA	100	30	3.2
Cefoxitin	0.9	30	33	NH	NH	NH	NA	NA	NA	NA	NA	NA	<1	NA	NA	NA	NA	NA
Cefuroxime	NA	NA	NA	NA	NA	NA	NA	NA	NA	2,000	24	230	NA	NA	NA	NA	NA	NA
Ceftazidime	380	2,000	188	ND	>3,000	ND	70	3,519	19.9	NA	NA	NA	10	NA	NA	NA	NA	NA
Cefotaxime	68	4,600	15	2.2	890	2.5	43	652	66	4,300	38	314	300	NA	NA	7	26	0.2
Cefepime	2.8	1,800	1.6	1.1	1900	0.6	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Cefpirome	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	20	151	0.14
Imipenem	0.003	45	0.07	0.004	0.45	NA	NA	NA	NA	NA	NA	NA	<1	NA	NA	NA	NA	NA
Meropenem	NH	NH	NH	NH	NH	NH	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Aztreonem	NH	NH	NH	NH	NH	NH	43	442	97.3	400	500	2	<1	NA	NA	NA	NA	NA

[a] Data were adapted from references 5, 36, 40, 41, and 45. k_{cat} values are available only for GES-1 and GES-2. V_{max} values are relative to that of benzylpenicillin and, for VEB-1, TEM-4 and SHV2a, relative to that of cephaloridine, which were set equal to 100. Abbreviations: NA, data not available; NH, not hydrolyzed (the initial rate of hydrolysis is reported to be lower than 0.001 $\mu M^{-1}\ s^{-1}$); ND, not determinable due to K_m values that are too high.

toward the narrow-spectrum penicillins and carbapenems (45), notably, imipenem (Table 3). GES-2 has a higher affinity for imipenem than GES-1 does (Table 3) (45). Although the rate of hydrolysis of imipenem by GES-2 is marginal compared to those of class B enzymes (45), GES-2 may confer resistance to imipenem, most likely when it is associated with a membrane impermeability-mediated resistance mechanism (45). Studies of inhibition by GES-2 revealed a marked increase in its 50% inhibitory concentration (IC_{50}) for imipenem compared to the IC_{50} of GES-1 (8 ± 2 and 0.1 μM, respectively) (45). The IC_{50} of GES-1 for clavulanic acid compared to that of GES-2 reveals a difference of ca. 10^3 (IC_{50}s, 15 nM and 1 ± 0.5 μM, respectively) (45), which may indicate future selection of bla_{GES} derivatives with resistance to enzyme inhibitors.

The latest non-TEM non-SHV ESBL characterized in *P. aeruginosa* is IBC-2, reported in a Greek isolate (27). IBC-2 confers resistance to ceftazidime and other oxyimino-cephalosporins and is inhibited by imipenem, tazobactam, and clavulanic acid (27). IBC-2 differs from IBC-1 by one amino acid change, which occurs outside of the omega loop at Ambler position 104 (Glu-to-Lys substitution), with both enzymes being highly related to the GES-1 and GES-2 lineage (Fig. 1) (16, 27).

GENETIC DETERMINANTS

The genes encoding the TEM- and SHV-type enzymes are usually plasmid located in the *Enterobacteriaceae* (23). The spread of these plasmids may be limited by species-related plasmid replication. A plasmid location of genes encoding ESBLs of the TEM and SHV series has been reported for bla_{SHV-12}, bla_{TEM-24}, and bla_{TEM-42} in *P. aeruginosa* and simultaneously in enterobacterial isolates from the same patients (8, 25, 30). Recently, the bla_{TEM-21} gene was identified as part of a chromosome-located Tn*801* transposon disrupted by insertion of an IS*6100* element (13). Whereas bla_{VEB}-like genes are mostly plasmid encoded in the *Enterobacteriaceae*, they are mostly chromosome encoded in *P. aeruginosa* (17, 18). The same is true for the bla_{PER}-like genes (52–54), whereas the bla_{GES} and bla_{IBC} genes have been found to be either plasmid or chromosome encoded in *P. aeruginosa* (14, 27, 44) and have also been identified in the *Enterobacteriaceae* (40). However, in the latter case, epidemiological surveys are not yet available.

Along with a plasmid location, many antibiotic resistance genes have been identified as a form of gene cassettes and as part of class 1 integrons in *P. aeruginosa* (49). Whereas genes encoding β-lactamases of Ambler class B (metalloenzymes) and Ambler class D (oxacillinases) are usually located in class 1 integrons, genes encoding VEB- and GES-type enzymes are the only genes encoding class A ESBLs that are associated with these genetic determinants. Conversely, the bla_{PER-1} gene is not integron associated (35). In several cases, bla_{GES} and bla_{VEB} genes have been associated with integrons with other β-lactamase genes, bla_{OXA-5} and $bla_{OXA-10,}$ respectively (17, 43). The gene-associated sequences are almost identical for the bla_{GES} genes (the same is true for the bla_{VEB} genes), thus underlining in those cases the epidemic spread of gene cassettes.

Since several integrons have been reported to be transposon located, these structures may provide an additional means of mobility for these antibiotic resistance genes and may explain the plasmid and chromosomal locations of the same ESBL gene in *P. aeruginosa* (13). Future work will be directed to the identification of the transposon structures that contain these integrons in *P. aeruginosa*. The bla_{VEB-1} gene has been identified within a composite transposon in *E. coli* (18), whereas known class 1 integrons are located on Tn*21* derivatives, which are commonly found in *Pseudomonas* spp. (22). Since bla_{VEB}-like and bla_{GES}-like genes are integron located, it is possible that their presence in *P. aeruginosa* may result from horizontal transfer from gram-negative aerobes (other than the *Enterobacteriaceae*), which are known to be sources of integrons, that may be present in the same ecologic niche.

TABLE 4. MICs of β-lactams for several representative class A ESBL-producing *P. aeruginosa* isolates[a]

β-Lactam[b]	MIC (μg/ml) for the following extended-spectrum β-lactamase (isolate denomination):						
	VEB-1 (JES-1)	PER-1 (RNL-1)	GES-1 (695)	GES-2 (GW-1)	SHV2a (RP-1)	TEM-4 (Stel)	IBC-2 (555)
Ticarcillin	>512	512	>512	>512	>512	>512	>256
Ticarcillin + CLA	256	256	64	>512	64	32	>256
Piperacillin	128	32	512	128	256	32	>256
Piperacillin + TZB	NA[c]	NA	64	128	16	8	>256
Ceftazidime	512	128	32	32	32	8	>256
Ceftazidime + CLA	128	4	32	16	8	4	NA
Cefotaxime	NA	64	NA	128	>512	128	>256
Cefepime	128	NA	16	32	NA	8	NA
Imipenem	32	0.5	1	16	2	4	>128
Meropenem	8	NA	NA	16	NA	1	>128
Aztrenom	>256	256	4	16	32	16	32

[a] Data were adapted from references 14, 27, 30 to 32, 34, 42, and 45.
[b] CLA, clavulanic acid at a fixed concentration of 2 μg/ml; TZB, tazobactam at a fixed concentration of 4 μg/ml.
[c] NA, data not available.

DETECTION

The presence of ESBLs in *P. aeruginosa* may be suspected in the face of an antibiotic resistance phenotype combining resistance to ticarcillin and ceftazidime and susceptibility to ticarcillin plus clavulanic acid (Table 4). Detection of ESBLs by double-disk synergy tests with clavulanate and extended-spectrum cephalosporins are sensitive and specific for the detection of ESBLs in the *Enterobacteriaceae* (7, 15). However, the same test may not be as useful for the detection of ESBLs in *P. aeruginosa* (11). These difficulties stem from several factors: (i) false-negative results due to naturally occurring β-lactamases, such as chromosome-encoded AmpCs that may be overexpressed; (ii) the simultaneous presence of metalloenzymes with carbapenem-hydrolyzing activities (the IMP and VIM series [12, 26]) or with extended-spectrum oxacillinases (OXA-2 and OXA-10 derivatives and OXA-18) (17, 38); (iii) relative resistance to inhibition by clavulanate, as exemplified by GES-2 (45); and (iv) combined mechanisms of resistance, such as impermeability and efflux.

Our experience indicates that positive results by the double-disk synergy test are quite easily obtained with VEB-1- and PER-1-positive strains, whereas the synergy patterns may be more difficult to detect with GES-type enzymes (personal data). In several cases, the synergy image with TEM and SHV ESBLs may be hardly visible for *P. aeruginosa*. Synergy between imipenem and ceftazidime may be observed with bla_{GES}-like and bla_{PER-1} enzymes, as shown in Fig. 2. This synergy may be obscured in some cases by the induction effect of imipenem on the expression of the chromosomal cephalosporinase, resulting in a concomitant line of antagonism between ceftazidime- and imipenem-containing disks. This effect can be overcome to some extent by performing the double-disk synergy test with oxacillin-containing agar plates, since oxacillin inhibits the activities of Ambler class C enzymes (Fig. 2) (6).

When an ESBL is suspected in *P. aeruginosa*, PCR-based molecular techniques may help to identify the gene. The quality of the whole-cell DNA used as the template is an important factor for avoiding false-negative results (personal data). Standard PCR conditions with a series of primers designed for detection of the class A β-lactamase genes bla_{TEM}, bla_{SHV}, bla_{PER}, bla_{VEB}, and $bla_{GES/IBC}$ could be used (Table 5). How-

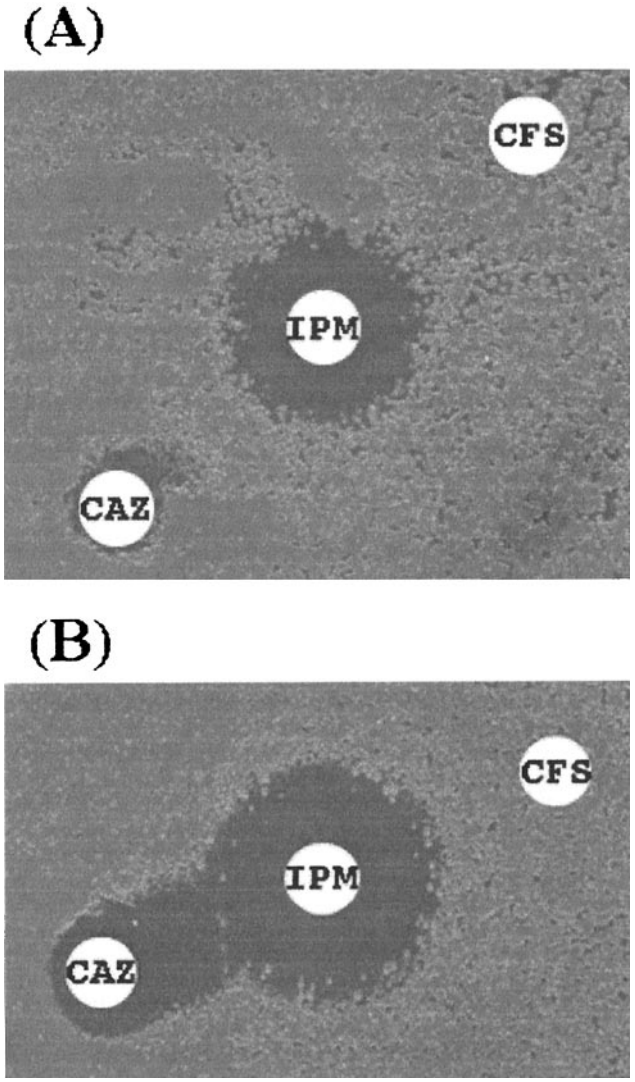

FIG. 2. Double-disk synergy test performed with imipenem (IPM)- and ceftazidime (CAZ)-containing disks and a GES-1-producing *P. aeruginosa* isolate without (A) or with (B) cloxacillin (200 μg/ml)-containing Mueller-Hinton agar plates. No synergy was visible with a cefsulodin (CFS)-containing disk.

TABLE 5. Primers used for detection of genes encoding class A ESBLs in *P. aeruginosa*

Primer name	Sequence (5′ to 3′)	Gene detected	Reference(s)
VEB-1A	CGACTTCCATTTCCCGATGC	bla_{VEB}	43
VEB-1B	GGACTCTGCAACAAATACGC		
PER-A	ATGAATGTCATTATAAAAGC	bla_{PER}	10, 45
PER-B	AATTTGGGCTTAGGGCAGAA		
GES-1A	ATGCGCTTCATTCACGCAC	bla_{GES}	45
GES-1B	CTATTTGTCCGTGCTCAGG		
TEM-A	GAGTATTCAACATTTCCGTGTC	bla_{TEM}	45
TEM-B	TAATCAGTGAGGCACCTATCTC		
SWSHV-A	AAGATCCACTATCGCCAGCAG	bla_{SHV}	31
SWSHV-B	ATTCAGTTCCGTTTCCCAGCGG		

ever, PCR experiments without further sequencing of the PCR products cannot differentiate between narrow-spectrum and extended-spectrum enzymes of the TEM and SHV series (21). Other methods such as isoelectric focusing analysis may only indicate the presence of acquired β-lactamases rather than identify an ESBL precisely. For example, PER-1 and narrow-spectrum TEM-1 enzymes share identical pI values of 5.4 (34). Primers designed to anneal to the ends of class 1 integrons may also help in the retrieval of PCR products that may contain ESBL genes. Nucleotide sequence analysis of PCR products, whether or not it is combined with other methods (23), is still the only acceptable way to accurately discriminate between ESBL genes of the same family.

CLINICAL CONSEQUENCES

The most appropriate antibiotic regimen for the treatment of infections due to ESBL-positive *P. aeruginosa* strains remains to be determined due to the few clinical studies that have been conducted in this field of research. Three reports detail the antibiotic therapy and outcomes for patients infected with ESBL-positive *P. aeruginosa* isolates (17, 24, 44). A study of experimental pneumonia in rats caused by a PER-1-producing *P. aeruginosa* strain (29) indicated that a combination of amikacin and imipenem was synergistic against an imipenem- and amikacin-susceptible strain. As predicted by the results of in vitro susceptibility testing, cefepime and piperacillin-tazobactam exhibited marked inoculum effects in vivo (29). As previously documented for ESBL-producing strains of the *Enterobacteriaceae* (47), these results indicate that infections due to PER-1-producing *P. aeruginosa* strains would not be treated safely with piperacillin-tazobactam or cefepime alone (29). A population-based cohort study conducted with PER-1-producing *P. aeruginosa* strains in Turkey (52) identified the following factors as independent predictors of a poor clinical outcome: (i) impaired consciousness, (ii) male sex, and (iii) urinary tract infection. Other clinically significant variables in that study were the presence of a central venous catheter, the acquisition of the infection in an intensive care unit (ICU), and hypotension. Unfortunately, the authors did not comment on the antibiotic regimen used in that study (52). Clinical experience with VEB-1-positive *P. aeruginosa* strains may indicate the efficacy of carbapenem-containing antibiotic regimens as the target therapy (17).

In the single documented outbreak involving GES-2-producing *P. aeruginosa* strains, a total mortality rate of 62.5% (5 of 8 patients) was recorded (44). However, it is difficult to deduce the optimum antibiotic regimen to be given, since all of the patients had different underlying diseases. The outbreak was terminated by (i) increasing the hygiene and housekeeping measures in ICUs, (ii) restricting the movements of patients infected or colonized with multiple-drug-resistant *P. aeruginosa* isolates, and (iii) increasing the turnover of patients hospitalized in these ICUs (G. F. Weldhagen, personal data). The use of topical nonabsorbable antibiotics given orally, such as colistin (50), to control enteric reservoirs of ESBL-positive enterobacterial isolates has not been evaluated in the case of ESBL-positive *P. aeruginosa* strains.

MIC results may help in choosing the optimal antibiotic regimen, but susceptibility in vitro does not always guarantee success in vivo (Table 4). If the ESBL-positive isolate remains susceptible to carbapenems, the use of a carbapenem in combination with an antibiotic molecule of another non-β-lactam class should be proposed. Meropenem, unlike imipenem, remains stable to the hydrolytic activities of all class A ESBLs, including the β-lactamase GES-2 (45). Colonized skin wounds should not be treated with systemic antibiotics but, rather, should be dressed by topical application of antiseptics. Increased general hygiene measures in the hospital, as reported for the control of outbreaks caused by ESBL-positive enterobacterial isolates, are crucial in controlling outbreaks due to ESBL-positive *P. aeruginosa.*

CONCLUSION

Some ESBL-producing *P. aeruginosa* strains seem to be highly prevalent in certain geographic locations, such as those that produce the VEB and PER enzymes in Southeast Asia and Turkey, respectively. The detection of other ESBLs in other countries may, however, reflect laboratory research interest rather than the true distribution of these enzymes in *P. aeruginosa*.

Difficulties in laboratory detection of ESBLs and thus underreporting may likely increase the incidence and the prevalence of these enzymes worldwide, especially in developing countries. In several cases, the current high prevalence of ESBLs in *P. aeruginosa* in those countries may be the source for the transfer of ESBL-producing *P. aeruginosa* to developed Western countries, as well as a hidden reservoir for the transfer of ESBL genes to other gram-negative aerobes. Since *P. aeruginosa* is known to be a formidable pathogen in terms of the acquisition of additional resistance mechanisms, one should be aware that a multidrug resistance trend will be very difficult to reverse in this species. The reporting of the β-lactamase GES-2, which is a weak carbapenem-hydrolyzing β-lactamase, raises the additional threat of the selection of β-lactamases with very broad substrate profiles and increased levels of resistance to inhibitors from ESBLs.

Reports of structurally related, integron-located ESBL genes in *P. aeruginosa* strains from different parts of the world add novel steps in the saga of the evolutionary transfer of

β-lactamase-mediated antibiotic resistance, with questions arising as to the origins of these genes. Additionally, coresistance and the coexpression of resistance determinants as the result of their integron location may stabilize further nonrelated antibiotic resistance genes. In other words, antibiotic regimens that may contain rifampin (a rifampin resistance gene has been associated with bla_{VEB-1} [51]) or aminoglycosides, for example, may enhance the prevalence of genes encoding resistance to structurally unrelated antibiotic molecules, including expanded-spectrum cephalosporins and even carbapenems (e.g., in β-lactamase GES-2). Thus, changes in antibiotic use policies may apply not only to extended-spectrum cephalosporins but also to non-β-lactam antibiotics.

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REFERENCES

1. **Ambler, R. P., A. F. W. Coulson, J.-M. Frère, J. M. Ghuysen, B. Joris, M. Forsman, R. C. Lévesque, G. Tirabi, and S. G. Waley.** 1991. A standard numbering scheme for the class A β-lactamases. Biochem. J. **276:**269–272.
2. **Ben-Mahrez, K., S. Rejiba, C. Belhadj, and O. Belhadj.** 1999. β-Lactamase mediated resistance to extended-spectrum cephalosporins among clinical isolates of *Pseudomonas aeruginosa*. Res. Microbiol. **150:**403–406.
3. **Bert, F., C. Branger, and N. Lambert-Zechowsky.** 2002. Identification of PSE and OXA β-lactamase genes in *Pseudomonas aeruginosa* using PCR-restriction fragment length polymorphism. J. Antimicrob. Chemother. **50:**11–18.
4. **Bonnet, R., J. L. Sampaio, C. Chanal, D. Sirot, C. De Champs, J. L. Viellard, R. Labia, and J. Sirot.** 2000. A novel class A extended-spectrum β-lactamase (BES-1) in *Serratia marcescens* in Brazil. Antimicrob. Agents Chemother. **44:**3061–3068.
5. **Bouthors, A.-T., N. Dagoneau, S. Blanchard, T. Naas, P. Nordmann, V. Jarlier, and W. Sougakoff.** 1998. Role of residues 104, 164, 166, 238, 240 in the substrate profile of PER-1 β-lactamase hydrolysing third-generation cephalosporins. Biochem. J. **330:**1443–1449.
6. **Bush, K., G. A. Jacoby, and A. A. Medeiros.** 1995. A functional classification scheme for β-lactamases and its correlation with molecular structure. Antimicrob. Agents Chemother. **39:**1211–1233.
7. **Carter, M. W., K. J. Oakton, M. Warner, and D. M. Livermore.** 2000. Detection of extended-spectrum β-lactamases in *Klebsiellae* with the Oxoid combination disk method. J. Clin. Microbiol. **38:**4228–4232.
8. **Chanawong, A., F. H. M′Zali, J. Heritage, A. Lulitanond, and P. M. Hawkey.** 2001. SHV-12, SHV-5, SHV-2a and VEB-1 extended-spectrum β-lactamases in gram-negative bacteria isolated in a university hospital in Thailand. J. Antimicrob. Chemother. **48:**839–852.
9. **Chen, H. Y., M. Yuan, and D. M. Livermore.** 1995. Mechanisms of resistance to β-lactam antibiotics amongst *Pseudomonas aeruginosa* isolates collected in the UK in 1993. J. Med. Microbiol. **43:**300–309.
10. **Claeys, G., G. Verschraegen, T. De Baere, and M. Vaneechoutte.** 2000. PER-1 β-lactamase producing *Pseudomonas aeruginosa* in an intensive care unit. J. Antimicrob. Chemother. **45:**924–925.
11. **De Champs, C., L. Poirel, R. Bonnet, D. Sirot, C. Chanal, J. Sirot, and P. Nordmann.** 2002. Prospective survey of β-lactamases produced by ceftazidime-resistant *Pseudomonas aeruginosa* isolated in a French hospital in 2000. Antimicrob. Agents Chemother. **46:**3031–3034.
12. **Docquier, J.-D., F. Luzzaro, G. Amicosante, A. Toniolo, and G. M. Rossolini.** 2001. Multidrug-resistant *Pseudomonas aeruginosa* producing PER-1 extended-spectrum serine β-lactamase and VIM-2 metallo-β-lactamase. Emerg. Infect. Dis. **7:**910–911.
13. **Dubois, V., C. Arpin, P. Noury, and C. Quentin.** 2002. Clinical strain of *Pseudomonas aeruginosa* carrying a bla_{TEM-21} gene located on a chromosomal interrupted Tn*4* type transposon. Antimicrob. Agents Chemother. **46:**3624–3626.
14. **Dubois, V., L. Poirel, C. Marie, C. Arpin, P. Nordmann, and C. Quentin.** 2002. Molecular characterization of a novel class 1 integron containing bla_{GES-1} and a fused product of *aac(3)-Ib*/*aac(6′)-Ib′* gene cassettes in *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother. **46:**638–645.
15. **Florijn, A., S. Nijssen, F.-J. Schmitz, J. Verhoef, and A. C. Fluit.** 2002. Comparison of E-test and double disk diffusion test for detection of extended spectrum β-lactamases. Eur. J. Clin. Microbiol. Infect. Dis. **21:**241–243.
16. **Giakkoupi, P., L. S. Tzouvelekis, A. Tsakris, V. Loukova, D. Sofianou, and E. Tzelepi.** 2000. IBC-1, a novel integron-associated class A β-lactamase with extended-spectrum properties produced by an *Enterobacter cloacae* clinical strain. Antimicrob. Agents Chemother. **44:**2247–2253.
17. **Girlich, D., T. Naas, A. Leelaporn, L. Poirel, M. Fennewald, and P. Nordmann.** 2002. Nosocomial spread of the integron-located *veb-1*-like cassette encoding an extended-spectrum β-lactamase in *Pseudomonas aeruginosa* in Thailand. Clin. Infect. Dis. **34:**603–611.
18. **Girlich, D., L. Poirel, A. Leelaporn, A. Karim, C. Tribuddharat, M. Fennewald, and P. Nordmann.** 2001. Molecular epidemiology of the integron-located VEB-1 extended spectrum β-lactamase in nosocomial enterobacterial isolates in Bangkok, Thailand. J. Clin. Microbiol. **39:**175–182.
19. **Jarlier, V., M.-H. Nicolas, G. Fournier, and A. Philippon.** 1988. Extended broad-spectrum β-lactamases conferring transferable resistance to newer β-lactam agents in *Enterobacteriaceae*: hospital prevalence and susceptibility patterns. Rev. Infect. Dis. **10:**867–878.
20. **Langaee, T. Y., L. Gagnon, and A. Huletsky.** 2000. Inactivation of the *ampD* gene in *Pseudomonas aeruginosa* leads to moderate-basal-level and hyperinducible AmpC β-lactamase expression. Antimicrob. Agents Chemother. **44:**583–589.
21. **Lee, S. H., J. Y. Kim, S. H. Shin, S. K. Lee, M. M. Choi, I. Y. Lee, Y. B. Kim, J. Y. Cho, W. Jin, and K. J. Lee.** 2001. Restriction fragment length dimorphism-PCR method for the detection of extended-spectrum β-lactamases unrelated to TEM- and SHV-types. FEMS Microbiol. Lett. **200:**157–161.
22. **Liebert, C. A., R. M. Hall, and A. O. Summers.** 1999. Transposon Tn*21*, flagship of the floating genome. Microbiol. Mol. Biol. Rev. **63:**507–522.
23. **Livermore, D. M.** 1995. β-Lactamases in laboratory and clinical resistance. Clin. Microbiol. Rev. **8:**557–584.
24. **Luzzaro, F., E. Mantengoli, M. Perilli, G. Lombardi, V. Orlandi, A. Orsatti, G. Amicosante, G. M. Rossolini, and A. Toniolo.** 2001. Dynamics of a nosocomial outbreak of multidrug-resistant *Pseudomonas aeruginosa* producing the PER-1 extended-spectrum β-lactamase. J. Clin. Microbiol. **39:**1865–1870.
25. **Marchandin, H., H. Jean-Pierre, C. De Champs, D. Sirot, H. Darbas, P. F. Perigault, and C. Carrière.** 2000. Production of a TEM-24 plasmid-mediated extended-spectrum β-lactamase by a clinical isolate of *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother. **44:**213–216.
26. **Mavroidi, A., A. Tsakris, E. Tzelepi, S. Pournaras, V. Loukova, and L. S. Tzouvelekis.** 2000. Carbapenem-hydrolysing VIM-2 metallo-β-lactamase in *Pseudomonas aeruginosa* from Greece. J. Antimicrob. Chemother. **46:**1041–1042.
27. **Mavroidi, A., E. Tzelepi, A. Tsakris, V. Miriagou, D. Sofianou, and L. S. Tzouvelekis.** 2001. An integron-associated β-lactamase (IBC-2) from *Pseudomonas aeruginosa* is a variant of the extended-spectrum β-lactamase IBC-1. J. Antimicrob. Chemother. **48:**627–630.
28. **Medeiros, A. A.** 1997. Evolution and dissemination of β-lactamases accelerated by generations of β-lactam antibiotics. Clin. Infect. Dis. **24:**S19–S45.
29. **Mimoz, O., N. Elhelali, S. Léotard, A. Jacolot, F. Laurent, K. Samii, O. Petitjean, and P. Nordmann.** 1999. Treatment of experimental pneumonia in rats caused by a PER-1 extended-spectrum β-lactamase producing strain of *Pseudomonas aeruginosa*. J. Antimicrob. Chemother. **44:**91–97.
30. **Mugnier, P., P. Dubrous, I. Casin, G. Arlet, and E. Collatz.** 1996. A TEM-derived extended-spectrum β-lactamase in *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother. **40:**2488–2493.
31. **Naas, T., L. Philippon, L. Poirel, E. Ronco, and P. Nordmann.** 1999. An SHV-derived extended-spectrum β-lactamase in *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother. **43:**1281–1284.
32. **Naas, T., L. Poirel, A. Karim, and P. Nordmann.** 1999. Molecular characterization of In*50*, a class 1 integron encoding the gene for the extended-spectrum β-lactamase VEB-1 in *Pseudomonas aeruginosa*. FEMS Microbiol. Lett. **176:**411–419.
33. **Navarro, F., and E. Miro.** 2002. Update on CTX-M-type β-lactamases. Rev. Med. Microbiol. **13:**63–73.
34. **Nordmann, P., E. Ronco, T. Naas, C. Duport, Y. Michel-Briand, and R. Labia.** 1993. Characterization of a novel extended-spectrum β-lactamase from *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother. **37:**962–969.
35. **Nordmann, P., and T. Naas.** 1994. Sequence analysis of PER-1 extended-spectrum β-lactamase from *Pseudomonas aeruginosa* and comparison with class A β-lactamases. Antimicrob. Agents Chemother. **38:**104–114.
36. **Paul, G. C., G. Gerbaud, A. Buré, A. M. Philippon, B. Pangon, and P. Courvalin.** 1989. TEM-4, a new plasmid-mediated β-lactamase that hydrolyzes broad-spectrum cephalosporins in a clinical isolate of *Escherichia coli*. Antimicrob. Agents Chemother. **33:**1958–1963.
37. **Pellegrino, F. L., L. M. Teixeira, G. Carvalho, S. Aranha Nouer, M. Pinto De Oliveira, J. L. Mello Sampaio, A. D'Avilas Freitas, A. L. Ferreira, L. Amorim, L. W. Riley, and B. M. Moreira.** 2002. Occurrence of a multidrug-resistant *Pseudomonas aeruginosa* clone in different hospitals in Rio de Janeiro, Brazil. J. Clin. Microbiol. **40:**2420–2424.
38. **Philippon, L. N., T. Naas, A.-T. Bouthors, V. Barakett, and P. Nordmann.** 1997. OXA-18, a class D clavulanic acid-inhibited extended-spectrum β-lactamase from *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother. **41:**2188–2195.
39. **Podbielski, A., J. Schönling, B. Melzer, K. Warnatz, and H. G. Leusch.** 1991.

Molecular characterization of a new plasmid-encoded SHV-type β-lactamase (SHV-2 variant) conferring high-level cefotaxime resistance upon *Klebsiella pneumoniae*. J. Gen. Microbiol. **137:**569–578.

40. **Poirel, L., I. Le Thomas, T. Naas, A. Karim, and P. Nordmann.** 2000. Biochemical sequence analyses of GES-1, a novel class A extended-spectrum β-lactamase, and the class 1 integron In52 from *Klebsiella pneumoniae*. Antimicrob. Agents Chemother. **44:**622–632.
41. **Poirel, L., T. Naas, M. Guibert, E. B. Chaibi, R. Labia, and P. Nordmann.** 1999. Molecular and biochemical characterization of VEB-1, a novel class A extended-spectrum β-lactamase encoded by an *Escherichia coli* integron gene. Antimicrob. Agents Chemother. **43:**573–581.
42. **Poirel, L., E. Ronco, T. Naas, and P. Nordmann.** 1999. Extended-spectrum β-lactamase TEM-4 in *Pseudomonas aeruginosa*. Clin. Microbiol. Infect. **5:**651–652.
43. **Poirel, L., V. O. Rotimi, E. M. Mokaddas, A. Karim, and P. Nordmann.** 2001. VEB-1-like extended-spectrum β-lactamases in *Pseudomonas aeruginosa*, Kuwait. Emerg. Infect. Dis. **7:**468–470.
44. **Poirel, L., G. F. Weldhagen, C. De Champs, and P. Nordmann.** 2002. A nosocomial outbreak of *Pseudomonas aeruginosa* isolates expressing the extended spectrum β-lactamase GES-2 in South Africa. J. Antimicrob. Chemother. **49:**561–565.
45. **Poirel, L., G. F. Weldhagen, T. Naas, C. de Champs, M. G. Dove, and P. Nordmann.** 2001. GES-2, a class A β-lactamase from *Pseudomonas aeruginosa* with increased hydrolysis of imipenem. Antimicrob. Agents Chemother. **45:**2598–2603.
46. **Rejiba, S., F. Limam, C. Belhadj, O. Belhadj, and K. Ben-Mahrez.** 2002. Biochemical characterization of a novel extended-spectrum β-lactamase from *Pseudomonas aeruginosa* 802. Microb. Drug Resist. **8:**9–13.
47. **Rice, L. B., J. D. Yao, K. Klimm, G. M. Eliopoulos, and R. C. Moellering.** 1991. Efficacy of different β-lactams against an extended-spectrum β-lactamase-producing *Klebsiella pneumoniae* strain in the rat intra-abdominal abscess model. Antimicrob. Agents Chemother. **35:**1243–1244.
48. **Silva, J., C. Aguilar, G. Ayala, M. A. Estrada, U. Garza-Ramos, R. Lara-Lemus, and L. Ledezma.** 2000. TLA-1: a new plasmid-mediated extended-spectrum β-lactamase from *Escherichia coli*. Antimicrob. Agents Chemother. **44:**997–1003.
49. **Stokes, H. W., and R. M. Hall.** 1989. A novel family of potentially mobile DNA elements encoding site-specific gene-integration functions: integrons. Mol. Microbiol. **3:**1669–1683.
50. **Stoutenbeek, C. P.** 1989. The role of systemic antibiotic prophylaxis in infection prevention in intensive care by SDD. Infection **17:**418–421.
51. **Tribuddharat, C., and M. Fennewald.** 1999. Integron-mediated rifampin resistance in *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother. **43:** 960–962.
52. **Vahaboglu, H., F. Coskunkan, O. Tansel, R. Öztürk, N. Sahin, I. Koksal, B. Kocazeybek, M. Tatman-Ötkun, H. Leblebicioglu, M. A. Ozinel, H. Akalin, S. Kocagoz, and V. Korten.** 2001. Clinical importance of extended-spectrum β-lactamase (PER-1-type) producing *Acinetobacter* spp. and *Pseudomonas aeruginosa* strains. J. Med. Microbiol. **50:**642–645.
53. **Vahaboglu, H., S. Dodanli, C. Eroglu, R. Öztürk, G. Soyletir, I. Yildirim, and V. Akan.** 1996. Characterization of multiple-antibiotic resistant *Salmonella typhimurium* strains: molecular epidemiology of PER-1-producing isolates and evidence for nosocomial plasmid exchange by a clone. J. Clin. Microbiol. **34:**2942–2946.
54. **Vahaboglu, H., R. Öztürk, G. Aygun, F. Coskunkan, A. Yaman, A. Kaygusuz, H. Leblebicioglu, I. Balik, K. Aydin, and M. Otkün.** 1997. Widespread detection of PER-1-type extended-spectrum β-lactamases among nosocomial *Acinetobacter* and *Pseudomonas aeruginosa* isolates in Turkey: a nationwide multicenter study. Antimicrob. Agents Chemother. **41:**2265–2269.
55. **Wolska, M. K., K. Bukowski, and A. Jakubczak.** 2001. Occurrence of β-lactamase type ESBL and IBL in *Pseudomonas aeruginosa* rods. Med. Dosw. Mikrobiol. **53:**45–51.